Anti-MicroRNA Oligonucleotide Molecules

This application is a continuing application of U.S. Application Serial Number 10/778,908 filed on February 13, 2004. The specification of U.S. Application Serial Number 10/778,908 is hereby incorporated by reference in its entirety.

The invention claimed herein was made with the help of grant number 1 R01 GM068476-01 from NIH/NIGMS. The U.S. government has certain rights in the invention.

BACKGROUND OF THE INVENTION

RNA silencing is a fundamental mechanism of gene regulation that uses double-stranded RNA (dsRNA) derived 21- to 28-nucleotide (nt) small RNAs to guide mRNA degradation, control mRNA translation or chromatin modification. Recently, several hundred novel genes were identified in plants and animals that encode transcripts that contain short dsRNA hairpins.

Defined 22-nt RNAs, referred to as microRNAs (miRNAs), are reported to be excised by dsRNA specific endonucleases from the hairpin precursors. The miRNAs are incorporated into ribonucleoprotein particles (miRNPs).

Plant miRNAs target mRNAs containing sequence segments with high complementarity for degradation or suppress translation of partially complementary mRNAs. Animal miRNAs appear to act predominantly as translational repressors. However, animal miRNAs have also been reported to guide RNA degradation. This indicates that animal miRNPs act like small interfering RNA (siRNA)-induced silencing complexes (RISCs).

Understanding the biological function of miRNAs requires knowledge of their mRNA targets. Bioinformatic approaches have been used to predict mRNA targets, among which transcription factors and proapoptotic genes were prominent candidates. Processes such as *Notch* signaling, cell proliferation, morphogenesis and axon guidance appear to be controlled by miRNA genes.

Therefore, there is a need for materials and methods that can help elucidate the function of known and future microRNAs. Due to the ability of microRNAs to induce RNA degradation

or repress translation of mRNA which encode important proteins, there is also a need for novel compositions for inhibiting microRNA-indcued cleavage or repression of mRNAs.

SUMMARY THE INVENTION

In one embodiment, the invention provides an isolated single stranded anti-microRNA molecule comprising a minimum of ten moieties and a maximum of fifty moieties on a molecular backbone, the molecular backbone comprising backbone units, each moiety comprising a base bonded to a backbone unit, each base forming a Watson-Crick base pair with a complementary base wherein at least ten contiguous bases have the same sequence as a sequence of bases in any one of the anti-microRNA molecules shown in Tables 1-4, except that up to thirty percent of the bases pairs may be wobble base pairs, and up to 10% of the contiguous bases may be additions, deletions, mismatches, or combinations thereof; no more than fifty percent of the contiguous moieties contain deoxyribonuleotide backbone units; the moiety in the molecule at the position corresponding to position 11 of the microRNA is non-complementary; and the molecule is capable of inhibiting microRNP activity.

In another embodiment, the invention provides a method for inhibiting microRNP activity in a cell, the microRNP comprising a microRNA molecule, the microRNA molecule comprising a sequences of bases complementary of the sequence of bases in a single stranded anti-microRNA molecule, the method comprising introducing into the cell the single-stranded anti-microRNA molecule comprising a sequence of a minimum of ten moieties and a maximum of fifty moieties on a molecular backbone, the molecular backbone comprising backbone units, each moiety comprising a base bonded to a backbone unit, each base forming a Watson-Crick base pair with a complementary base, wherein at least ten contiguous bases of the anti-microRNA molecule are complementary to the microRNA, except that up to thirty percent of the bases may be substituted by wobble base pairs, and up to ten percent of the at least ten moieties may be additions, deletions, mismatches, or combinations thereof; no more than fifty percent of the contiguous moieties contain deoxyribonuleotide backbone units; and the moiety in the molecule at the position corresponding to position 11 of the microRNA is non-complementary.

In another embodiment, the invention provides an isolated microRNA molecule comprising a minimum of ten moieties and a maximum of fifty moieties on a molecular

backbone, the molecular backbone comprising backbone units, each moiety comprising a base bonded to a backbone unit, wherein at least ten contiguous bases have the same sequence as a sequence of bases in any one of the microRNA molecules shown in Table 2, except that up to thirty percent of the bases pairs may be wobble base pairs, and up to 10% of the contiguous bases may be additions, deletions, mismatches, or combinations thereof; and no more than fifty percent of the contiguous moieties contain deoxyribonuleotide backbone units.

In another embodiment, the invention provides an isolated microRNA molecule comprising a minimum of ten moieties and a maximum of fifty moieties on a molecular backbone, the molecular backbone comprising backbone units, each moiety comprising a base bonded to a backbone unit, wherein at least ten contiguous bases have any one of the microRNA sequences shown in Tables 1, 3 and 4, except that up to thirty percent of the bases pairs may be wobble base pairs, and up to 10% of the contiguous bases may be additions, deletions, mismatches, or combinations thereof; no more than fifty percent of the contiguous moieties contain deoxyribonuleotide backbone units; and is modified for increased nuclease resistance.

In yet another embodiment, the invention provides an isolated single stranded anti-microRNA molecule comprising a minimum of ten moieties and a maximum of fifty moieties on a molecular backbone, the molecular backbone comprising backbone units, each moiety comprising a base bonded to a backbone unit, each base forming a Watson-Crick base pair with a complementary base wherein at least ten contiguous bases have the same sequence as a sequence of bases in any one of the anti-microRNA molecules shown in Tables 1-4, except that up to thirty percent of the bases pairs may be wobble base pairs, and up to 10% of the contiguous bases may be additions, deletions, mismatches, or combinations thereof; no more than fifty percent of the contiguous moieties contain deoxyribonuleotide backbone units; and the molecule is capable of inhibiting microRNP activity.

In yet a further embodiment, the invention provides a method for inhibiting microRNP activity in a cell, the microRNP comprising a microRNA molecule, the microRNA molecule comprising a sequences of bases complementary of the sequence of bases in a single stranded anti-microRNA molecule, the method comprising introducing into the cell the single-stranded anti-microRNA molecule comprising a sequence of a minimum of ten moieties and a maximum

of fifty moieties on a molecular backbone, the molecular backbone comprising backbone units, each moiety comprising a base bonded to a backbone unit, each base forming a Watson-Crick base pair with a complementary base, wherein at least ten contiguous bases of the anti-microRNA molecule are complementary to the microRNA, except that up to thirty percent of the bases may be substituted by wobble base pairs, and up to ten percent of the at least ten moieties may be additions, deletions, mismatches, or combinations thereof; and no more than fifty percent of the contiguous moieties contain deoxyribonuleotide backbone units.

DESCRIPTION OF THE FIGURES

Figure 1 shows the modified nucleotide units discussed in the specification. B denotes any one of the following nucleic acid bases: adenosine, cytidine, guanosine, thymine, or uridine.

Figure 2. Antisense 2'-O-methyl oligoribonucleotide specifically inhibit miR-21 guided cleavage activity in HeLa cell S100 cytoplasmic extracts. The black bar to the left of the RNase T1 ladder represents the region of the target RNA complementary to miR-21. Oligonucleotides complementary to miR-21 were pre-incubated in S100 extracts prior to the addition of ^{32}P-cap-labelled cleavage substrate. Cleavage bands and T1 hydrolysis bands appear as doublets after a 1-nt slipping of the T7 RNA polymerase near the middle of the transcript indicated by the asterisk.

Figure 3. Antisense 2'-O-methyl oligoribonucleotides interfere with endogenous miR-21 RNP cleavage in HeLa cells. HeLa cells were transfected with pHcRed and pEGFP or its derivatives, with or without inhibitory or control oligonucleotides. EGFP and HcRed protein fluorescence were excited and recorded individually by fluorescence microscopy 24 h after transfection. Co-expression of co-transfected reporter plasmids was documented by superimposing of the fluorescence images in the right panel.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated single stranded anti-microRNA molecule. The molecule comprises a minimum number of ten moieties, preferably a minimum of thirteen, more preferably a minimum of fifteen, even more preferably a minimum of 18, and most preferably a minimum of 21 moieties.

The anti-microRNA molecule comprises a maximum number of fifty moieties, preferably a maximum of forty, more preferably a maximum of thirty, even more preferably a maximum of twenty-five, and most preferably a maximum of twenty-three moieties. A suitable range of minimum and maximum number of moieties may be obtained by combining any of the above minima with any of the above maxima.

Each moiety comprises a base bonded to a backbone unit. In this specification, a base refers to any one of the nucleic acid bases present in DNA or RNA. The base can be a purine or pyrimidine. Examples of purine bases include adenine (A) and guanine (G). Examples of pyrimidine bases include thymine (T), cytosine (C) and uracil (U). Each base of the moiety forms a Watson-Crick base pair with a complementary base.

Watson-Crick base pairs as used herein refers to the hydrogen bonding interaction between, for example, the following bases: adenine and thymine (A = T); adenine and uracil (A = U); and cytosine and guanine (C = G). The adenine can be replaced with 2,6-diaminopurine without compromising base-pairing.

The backbone unit may be any molecular unit that is able stably to bind to a base and to form an oligomeric chain. Suitable backbone units are well known to those in the art.

For example, suitable backbone units include sugar-phosphate groups, such as the sugar-phosphate groups present in ribonucleotides, deoxyribonucleotides, phosphorothioate deoxyribose groups, N'3-N'5 phosphoroamidate deoxyribose groups, 2'O-alkyl-ribose phosphate groups, 2'-O-alkyl-alkoxy ribose phosphate groups, ribose phosphate group containing a methylene bridge, 2'-Fluororibose phosphate groups, morpholino phosphoroamidate groups, cyclohexene groups, tricyclo phosphate groups, and amino acid molecules.

In one embodiment, the anti-microRNA molecule comprises at least one moiety which is a ribonucleotide moiety or a deoxyribonucleotide moiety.

In another embodiment, the anti-microRNA molecule comprises at least one moiety which confers increased nuclease resistance. The nuclease can be an exonuclease, an endonuclease, or both. The exonuclease can be a 3'→5' exonuclease or a 5'→3' exonuclease. Examples of 3'→5' human exonuclease include PNPT1, Werner syndrome helicase, RRP40,

RRP41, RRP42, RRP45, and RRP46. Examples of 5'→3' exonuclease include XRN2, and FEN1. Examples of endonucleases include Dicer, Drosha, RNase4, Ribonuclease P, Ribonuclease H1, DHP1, ERCC-1 and OGG1. Examples of nucleases which function as both an exonuclease and an endonuclease include APE1 and EXO1.

An anti-microRNA molecule comprising at least one moiety which confers increased nuclease resistance means a sequence of moieties wherein at least one moiety is not recognized by a nuclease. Therefore, the nuclease resistance of the molecule is increased compared to a sequence containing only unmodified ribonucleotide, unmodified deoxyribonucleotide or both. Such modified moieties are well known in the art, and were reviewed, for example, by Kurreck, Eur. J. Biochem. *270*, 1628-1644 (2003).

A modified moiety can occur at any position in the anti-microRNA molecule. For example, to protect the anti-microRNA molecule against 3'→5' exonucleases, the molecule can have at least one modified moiety at the 3' end of the molecule and preferably at least two modified moieties at the 3' end. If it is desirable to protect the molecule against 5'→3' exonuclease, the anti-microRNA molecule can have at least one modified moiety and preferably at least two modified moieties at the 5' end of the molecule. The anti-microRNA molecule can also have at least one and preferably at least two modified moieties between the 5' and 3' end of the molecule to increase resistance of the molecule to endonucleases. In one embodiment, all of the moieties are nuclease resistant.

In another embodiment, the anti-microRNA molecule comprises at least one modified deoxyribonucleotide moiety. Suitable modified deoxyribonucleotide moieties are known in the art.

A suitable example of a modified deoxyribonucleotide moiety is a phosphorothioate deoxyribonucleotide moiety. See structure 1 in figure 1. An anti-microRNA molecule comprising more than one phosphorothioate deoxyribonucleotide moiety is referred to as phosphorothioate (PS) DNA. See, for example, Eckstein, Antisense Nucleic Acids Drug Dev. *10*, 117-121 (2000).

Another suitable example of a modified deoxyribonucleotide moiety is an N'3-N'5 phosphoroamidate deoxyribonucleotide moiety. See structure 2 in figure 1. An oligonucleotide molecule comprising more than one phosphoroamidate deoxyribonucleotide moiety is referred to as phosphoroamidate (NP) DNA. See, for example, Gryaznov *et al.*, J. Am. Chem. Soc. *116*, 3143-3144 (1994).

In another embodiment, the molecule comprises at least one modified ribonucleotide moiety. Suitable modified ribonucleotide moieties are known in the art.

A suitable example of a modified ribonucleotide moiety is a ribonucleotide moiety that is substituted at the 2' position. The substituents at the 2' position may, for example, be a C_1 to C_4 alkyl group. The C_1 to C_4 alkyl group may be saturated or unsaturated, and unbranched or branched. Some examples of C_1 to C_4 alkyl groups include ethyl, isopropyl, and allyl. The preferred C_1 to C_4 alkyl group is methyl. See structure 3 in figure 1. An oligoribonucleotide molecule comprising more than one ribonucleotide moeity that is substituted at the 2' position with a C_1 to C_4 alkyl group is referred to as a 2'-O -(C_1-C_4 alkyl) RNA, e.g.,2'-O-methyl RNA (OMe RNA).

Another suitable example of a substituent at the 2' position of a modified ribonucleotide moiety is a C_1 to C_4 alkoxy - C_1 to C_4 alkyl group. The C_1 to C_4 alkoxy (alkyloxy) and C_1 to C_4 alkyl group may comprise any of the alkyl groups described above. The preferred C_1 to C_4 alkoxy - C_1 to C_4 alkyl group is methoxyethyl. See structure 4 in figure 1. An oligonucleotide molecule comprising more than one ribonucleotide moiety that is substituted at the 2' position with a C1 to C_4 alkoxy-C_1 to C_4 alkyl group is referred to as a 2'-O-(C_1 to C_4 alkoxy - C_1 to C_4 alkyl) RNA, e.g., 2'-O-methoxyethyl RNA (MOE RNA).

Another suitable example of a modified ribonucleotide moiety is a ribonucleotide that has a methylene bridge between the 2'-oxygen atom and the 4'-carbon atom. See structure 5 in figure 1. An oligoribonucleotide molecule comprising more than one ribonucleotide moiety that has a methylene bridge between the 2'-oxygen atom and the 4'-carbon atom is referred to as locked nucleic acid (LNA). See, for example, Kurreck *et al.*, Nucleic Acids Res. *30*, 1911-1918 (2002); Elayadi *et al.*, Curr. Opinion Invest. Drugs *2*, 558-561 (2001); Ørum *et al.*, Curr. Opinion Mol. Ther. *3*, 239-243 (2001); Koshkin *et al.*, Tetrahedron *54*, 3607-3630 (1998); Obika *et al.*,

Tetrahedron Lett.*39*, 5401-5404 (1998). Locked nucleic acids are commercially available from Proligo (Paris, France and Boulder, Colorado, USA).

Another suitable example of a modified ribonucleotide moiety is a ribonucleotide that is substituted at the 2' position with fluoro group. A modified ribonucleotide moiety having a fluoro group at the 2' position is a 2'-fluororibonucleotide moiety. Such moieties are known in the art. Molecules comprising more than one 2'-fluororibonucleotide moiety are referred to herein as 2'-fluororibo nucleic acids (FANA). See structure 7 in figure 1. Damha *et al.*, J. Am. Chem. Soc. *120*, 12976-12977 (1998).

In another embodiment, the anti-microRNA molecule comprises at least one base bonded to an amino acid residue. Moieties that have at least one base bonded to an amino acid residue will be referred to herein as peptide nucleic acid (PNA) moieties. Such moieties are nuclease resistance, and are known in the art. Molecules having more than one PNA moiety are referred to as peptide nucleic acids. See structure 6 in figure 1. Nielson, Methods Enzymol. *313*, 156-164 (1999); Elayadi, *et al*, *id.*; Braasch *et al.*, Biochemistry *41*, 4503-4509 (2002), Nielsen *et al.*, Science *254*, 1497-1500 (1991).

The amino acids can be any amino acid, including natural or non-natural amino acids. Naturally occurring amino acids include, for example, the twenty most common amino acids normally found in proteins, i.e., alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Glu), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ileu), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan, (Trp), tyrosine (Tyr), and valine (Val).

The non-natural amino acids may, for example, comprise alkyl, aryl, or alkylaryl groups. Some examples of alkyl amino acids include α-aminobutyric acid, β-aminobutyric acid, γ-aminobutyric acid, δ-aminovaleric acid, and ε-aminocaproic acid. Some examples of aryl amino acids include ortho-, meta, and para-aminobenzoic acid. Some examples of alkylaryl amino acids include ortho-, meta-, and para-aminophenylacetic acid, and γ-phenyl-β-aminobutyric acid.

Non-naturally occurring amino acids also include derivatives of naturally occurring amino acids. The derivative of a naturally occurring amino acid may, for example, include the addition or one or more chemical groups to the naturally occurring amino acid.

For example, one or more chemical groups can be added to one or more of the 2', 3', 4', 5', or 6' position of the aromatic ring of a phenylalanine or tyrosine residue, or the 4', 5', 6', or 7' position of the benzo ring of a tryptophan residue. The group can be any chemical group that can be added to an aromatic ring. Some examples of such groups include hydroxyl, C_1-C_4 alkoxy, amino, methylamino, dimethylamino, nitro, halo (i.e., fluoro, chloro, bromo, or iodo), or branched or unbranched C_1-C_4 alkyl, such as methyl, ethyl, n-propyl, isopropyl, butyl, isobutyl, or t-butyl.

Furthermore, other examples of non-naturally occurring amino acids which are derivatives of naturally occurring amino acids include norvaline (Nva), norleucine (Nle), and hydroxyproline (Hyp).

The amino acids can be identical or different from one another. Bases are attached to the amino acid unit by molecular linkages. Examples of linkages are methylene carbonyl, ethylene carbonyl and ethyl linkages. (Nielsen et al., *Peptide Nucleic Acids-Protocols and Applications*, Horizon Scientific Press, pages 1-19; Nielsen et al., *Science* 254: 1497-1500.)

One example of a PNA moiety is N-(2-aminoethyl)-glycine. Further examples of PNA moieties include cyclohexyl PNA, retro-inverso, phosphone, propionyl and aminoproline PNA.

PNA can be chemically synthesized by methods known in the art, e.g. by modified Fmoc or tBoc peptide synthesis protocols. The PNA has many desirable properties, including high melting temperatures (Tm), high base-pairing specificity with nucleic acid and an uncharged molecular backbone. Additionally, the PNA does not confer RNase H sensitivity on the target RNA, and generally has good metabolic stability.

Peptide nucleic acids are also commercially available from Applied Biosystems (Foster City, California, USA).

In another embodiment, the anti-microRNA molecule comprises at least one morpholino phosphoroamidate nucleotide moiety. A morpholino phosphoroamidate nucleotide moiety is a modified moiety which is nuclease resistant. Such moieties are known in the art. Molecules comprising more than one morpholino phosphoroamidate nucleotide moiety are referred to as morpholino (MF) nucleic acids. See structure 8 in figure 1. Heasman, Dev. Biol. *243*, 209-214 (2002). Morpholono oligonucleotides are commercially available from Gene Tools LLC (Corvallis, Oregon, USA).

In another embodiment, the anti-microRNA molecule comprises at least one cyclohexene nucleotide moiety. A cyclohexene nucleotide moiety is a modified moiety which is nuclease resistant. Such moieties are known in the art. Molecules comprising more than one cyclohexene nucleotide moiety are referred to as cyclohexene nucleic acids (CeNA). See structure 10 in figure 1. Wang *et al.*, J. Am. Chem. Soc. *122*, 8595-8602 (2000), Verbeure *et al.*, Nucleic Acids Res. *29*, 4941-4947 (2001).

In another embodiment, the anti-microRNA molecule comprises at least one tricyclo nucleotide moiety. A tricyclo nucleotide moiety is a modified moiety which is nuclease resistant. Such moieties are known in the art. Steffens *et al.*, J. Am. Chem. Soc. *119*, 11548-11549 (1997), Renneberg *et al.*, J. Am. Chem. Soc. *124*, 5993-6002 (2002). Molecules comprising more than one tricyclo nucleotide moiety are referred to as tricyclo nucleic acids (tcDNA). See structure 9 in figure 1.

In another embodiment, to increase nuclease resistance of the anti-microRNA molecules of the present invention to exonucleases, inverted nucleotide caps can be attached to the 5' end, the 3' end, or both ends of the molecule. An inverted nucleotide cap refers to a 3'→5' sequence of nucleic acids attached to the anti-microRNA molecule at the 5' and/or the 3' end. There is no limit to the maximum number of nucleotides in the inverted cap just as long as it does not interfere with binding of the anti-microRNA molecule to its target microRNA. Any nucleotide can be used in the inverted nucleotide cap. Typically, the inverted nucleotide cap is one nucleotide in length. The nucleotide for the inverted cap is generally thymine, but can be any nucleotide such as adenine, guanine, uracil, or cytosine.

Alternatively, an ethylene glycol compound and/or amino linkers can be attached to the either or both ends of the anti-microRNA molecule. Amino linkers can also be used to increase nuclease resistance of the anti-microRNA molecules to endonucleases. The table below lists some examples of amino linkers. The below listed amino linker are commercially available from TriLink Biotechnologies, San Diego, CA.

2'-Deoxycytidine-5-C6 Amino Linker (3' Terminus)
2'-Deoxycytidine-5-C6 Amino Linker (5' or Internal)
3' C3 Amino Linker
3' C6 Amino Linker
3' C7 Amino Linker
5' C12 Amino Linker
5' C3 Amino Linker
5' C6 Amino Linker
C7 Internal Amino Linker
Thymidine-5-C2 Amino Linker (5' or Internal)
Thymidine-5-C6 Amino Linker (3' Terminus)
Thymidine-5-C6 Amino Linker (Internal)

Chimeric anti-microRNA molecules containing a mixture of any of the moieties mentioned above are also known, and may be made by methods known, in the art. See, for example, references cited above, and Wang *et al*, Proc. Natl. Acad. Sci. USA *96*, 13989-13994 (1999), Liang *et al.*, Eur. J. Biochem. *269*, 5753-5758 (2002), Lok *et al.*, Biochemistry *41*, 3457-3467 (2002), and Damha *et al.*, J. Am. Chem. Soc. *120*, 12976-12977 (2002).

The molecules of the invention comprise at least ten contiguous, preferably at least thirteen contiguous, more preferably at least fifteen contiguous, and even more preferably at least twenty contiguous bases that have the same sequence as a sequence of bases in any one of the anti-microRNA molecules shown in Tables 1-4. The anti-microRNA molecules optimally

comprise the entire sequence of any one of the anti-microRNA molecule sequences shown in Tables 1-4.

For the contiguous bases mentioned above, up to thirty percent of the base pairs may be substituted by wobble base pairs. As used herein, wobble base pairs refers to either: i) substitution of a cytosine with a uracil, or 2) the substitution of a adenine with a guanine, in the sequence of the anti-microRNA molecule. These wobble base pairs are generally referred to as UG or GU wobbles. Below is a table showing the number of contiguous bases and the maximum number of wobble base pairs in the anti-microRNA molecule:

Table for Number of Wobble Bases

No. of Contiguous Bases	10	11	12	13	14	15	16	17	18
Max. No. of Wobble Base Pairs	3	3	3	3	4	4	4	5	5

No. of Contiguous Bases	19	20	21	22	23
Max. No. of Wobble Base Pairs	5	6	6	6	6

Further, up to ten percent, and preferably up to five percent of the contiguous bases can be additions, deletions, mismatches or combinations thereof. Additions refer to the insertion in the contiguous sequence of any moiety described above comprising any one of the bases described above. Deletions refer to the removal of any moiety present in the contiguous sequence. Mismatches refer to the substitution of one of the moieties comprising a base in the contiguous sequence with any of the above described moieties comprising a different base.

The additions, deletions or mismatches can occur anywhere in the contiguous sequence, for example, at either end of the contiguous sequence or within the contiguous sequence of the anti-microRNA molecule. If the contiguous sequence is relatively short, such as from about ten

to about 15 moieties in length, preferably the additions, deletions or mismatches occur at the end of the contiguous sequence. If the contiguous sequence is relatively long, such as a minimum of sixteen contiguous sequences, then the additions, deletions, or mismatches can occur anywhere in the contiguous sequence. Below is a table showing the number of contiguous bases and the maximum number of additions, deletions, mismatches or combinations thereof:

Table for Up to 10%

No. of Contiguous Bases	10	11	12	13	14	15	16	17	18
Max. No. of Additions, Deletions and/or Mismatches	1	1	1	1	1	1	1	1	1

No. of Contiguous Bases	19	20	21	22	23
Max. No. of Additions, Deletions and/or Mismatches	1	2	2	2	2

Table for Up to 5%

No. of Contiguous Bases	10	11	12	13	14	15	16	17	18
Max. No. of Additions, Deletions and/or Mismatches	0	0	0	0	0	0	0	0	0

No. of Contiguous Bases	19	20	21	22	23
Max. No. of Additions, Deletions and/or Mismatches	0	1	1	1	1

Furthermore, no more than fifty percent, and preferably no more than thirty percent, of the contiguous moieties contain deoxyribonucleotide backbone units. Below is a table showing the number of contiguous bases and the maximum number of deoxyribonucleotide backbone units:

Table for Fifty Percent Deoxyribonucleotide Backbone Units

No. of Contiguous Bases	10	11	12	13	14	15	16	17	18
Max. No. of Deoxyribonucleotide Backbone Units	5	5	6	6	7	7	8	8	9

No. of Contiguous Bases	19	20	21	22	23
Max. No. of Deoxyribonucleotide Backbone Units	9	10	10	11	11

Table for Thirty Percent Deoxyribonucleotide Backbone Units

No. of Contiguous Bases	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Max. No. of Deoxyribonucleotide Backbone Units	3	3	3	3	4	4	4	5	5	5	6	6	6	6

The moiety in the anti-RNA molecule at the position corresponding to position 11 of the microRNA is optionally non-complementary to a microRNA. The moiety in the anti-microRNA molecule corresponding to position 11 of the microRNA can be rendered non-complementary by an addition, deletion or mismatch as described above.

In another embodiment, if the anti-microRNA molecule comprises only unmodified moieties, then the anti-microRNA molecules comprises at least one base, in the at least ten contiguous bases, which is non-complementary to the microRNA and/or comprises an inverted nucleotide cap, ethylene glycol compound or an amino linker.

In yet another embodiment, if the at least ten contiguous bases in an anti-microRNA molecule is perfectly (i.e., 100%) complementary to ten contiguous bases in a microRNA, then the anti-microRNA molecule contains at least one modified moiety in the at least ten contiguous bases and/or comprises an inverted nucleotide cap, ethylene glycol compound or an amino linker.

As stated above, the maximum length of the anti-microRNA molecule is 50 moieties. Any number of moieties having any base sequence can be added to the contiguous base sequence. The additional moieties can be added to the 5' end, the 3' end, or to both ends of the contiguous sequence.

MicroRNA molecules are derived from genomic loci and are produced from specific microRNA genes. Mature microRNA molecules are processed from precursor transcripts that form local hairpin structures. The hairpin structures are typically cleaved by an enzyme known as Dicer, which generates one microRNA duplex. See Bartel, Cell 116, 281-297 (2004) for a review on microRNA molecules. The article by Bartel is hereby incorporated by reference.

Each strand of a microRNA is packaged in a microRNA ribonucleoprotein complex (microRNP). A microRNP in, for example, humans, also includes the proteins eIF2C2, the helicase Gemin3, and Gemin 4.

The sequence of bases in the anti-microRNA molecules of the present invention can be derived from a microRNA from any species e.g. such as a fly (e.g., *Drosophila melanogaster*), a worm (e.g., *C. elegans*). Preferably the sequence of bases is found in mammals, especially humans (*H. sapiens*), mice (e.g., *M. musculus*), and rats (*R. norvegicus*).

The anti-microRNA molecule is preferably isolated, which means that it is essentially free of other nucleic acids. Essentially free from other nucleic acids means that it is at least 90%, preferably at least 95% and, more preferably, at least 98% free of other nucleic acids.

Preferably, the molecule is essentially pure, which means that the molecules is free not only of other nucleic acids, but also of other materials used in the synthesis of the molecule, such as, for example, enzymes used in the synthesis of the molecule. The molecule is at least 90% free, preferably at least 95% free and, more preferably, at least 98% free of such materials.

The anti-microRNA molecules of the present invention are capable of inhibiting microRNP activity, preferable in a cell. Inhibiting microRNP activity refers to the inhibition of cleavage of the microRNA's target sequence or the repression of translation of the microRNA's target sequence. The method comprises introducing into the cell a single-stranded microRNA molecule.

Any anti-microRNA molecule can be used in the methods of the present invention, as long as the anti-microRNA is complementary, subject to the restrictions described above, to the microRNA present in the microRNP. Such anti-microRNAs include, for example, the anti-

microRNA molecules mentioned above (see Table 1-4), and the anti-microRNAs molecules described in international PCT application number WO 03/029459 A2, the sequences of which are incorporated herein by reference.

The invention also includes any one of the microRNA molecules having the sequences as shown in Table 2. The novel microRNA molecules in Table 2 may optionally be modified as described above for anti-microRNA molecules. The other microRNA molecules in Tables 1, 3 and 4 are modified for increased nuclease resistance as described above for anti-microRNA molecules.

Utility

The anti-microRNA molecules and the microRNA molecules of the present invention have numerous *in vivo, in vitro,* and *ex vivo* applications.

For example, the anti-microRNA molecules and microRNA of the present invention may be used as a modulator of the expression of genes which are at least partially complementary to the anti-microRNA molecules and microRNA. For example, if a particular microRNA is beneficial for the survival of a cell, an appropriate isolated microRNA of the present invention may be introduced into the cell to promote survival. Alternatively, if a particular microRNA is harmful (e.g., induces apoptosis, induces cancer, etc.), an appropriate anti-microRNA molecule can be introduced into the cell in order to inhibit the activity of the microRNA and reduce the harm.

In addition, anti-microRNA molecules and/or microRNAs of the present invention can be introduced into a cell to study the function of the microRNA. Any of the anti-microRNA molecules and/or microRNAs listed above can be introduced into a cell for studying their function. For example, a microRNA in a cell can be inhibited with a suitable anti-microRNA molecule. The function of the microRNA can be inferred by observing changes associated with inhibition of the microRNA in the cell in order to inhibit the activity of the microRNA and reduce the harm.

The cell can be any cell which expresses microRNA molecules, including the microRNA molecules listed herein. Alternatively, the cell can be any cell transfected with an expression vector containing the nucleotide sequence of a microRNA.

Examples of cells include, but are not limited to, endothelial cells, epithelial cells, leukocytes (e.g., T cells, B cells, neutrophils, macrophages, eosinophils, basophils, dendritic cells, natural killer cells and monocytes), stem cells, hemopoietic cells, embryonic cells, cancer cells.

The anti-microRNA molecules or microRNAs can be introduced into a cell by any method known to those skilled in the art. Useful delivery systems, include for example, liposomes and charged lipids. Liposomes typically encapsulate oligonucleotide molecules within their aqueous center. Charged lipids generally form lipid- oligonucleotide molecule complexes as a result of opposing charges.

These liposomes-oligonucleotide molecule complexes or lipid- oligonucleotide molecule complexes are usually internalized by endocytosis. The liposomes or charged lipids generally comprise helper lipids which disrupt the endosomal membrane and release the oligonucleotide molecules.

Other methods for introducing an anti-microRNA molecule or a microRNA into a cell include use of delivery vehicles, such as dendrimers, biodegradable polymers, polymers of amino acids, polymers of sugars, and oligonucleotide-binding nanoparticles. In addition, pluoronic gel as a depot reservoir can be used to deliver the anti-microRNA oligonucleotide molecules over a prolonged period. The above methods are described in, for example, Hughes et al., Drug Discovery Today *6*, 303-315 (2001); Liang et al. Eur. J. Biochem. *269* 5753-5758 (2002); and Becker et al., In *Antisense Technology in the Central Nervous System* (Leslie, R.A., Hunter, A.J. & Robertson, H.A., eds), pp.147-157, Oxford University Press.

Targeting of an anti-microRNA molecule or a microRNA to a particular cell can be performed by any method known to those skilled in the art. For example, the anti-microRNA molecule or microRNA can be conjugated to an antibody or ligand specifically recognized by receptors on the cell.

The sequences of microRNA and anti-microRNA molecules are shown in Tables 1-4 below. Human sequences are indicated with the prefix "hsa." Mouse sequences are indicated with the prefix "mmu." Rat sequences are indicated with the prefix "rno." *C. elegan* sequences are indicated with the prefix "cel." Drosophila sequences are indicated with the prefix "dme."

Table 1: Human, Mouse and Rat microRNA and anti-microRNA sequences.

microRNA name	microRNA sequence (5' to 3')	Anti-microRNA molecule sequence (5' to 3')
hsa-miR-100	AACCCGUAGAUCCGAACUUGUG	CACAAGUUCGGAUCUACGGGUU
hsa-miR-103	AGCAGCAUUGUACAGGGCUAUG	CAUAGCCCUGUACAAUGCUGCU
hsa-miR-105-5p	UCAAAUGCUCAGACUCCUGUGG	CCACAGGAGUCUGAGCAUUUGA
hsa-miR-106a	AAAAGUGCUUACAGUGCAGGUA	UACCUGCACUGUAAGCACUUUU
hsa-miR-106b	UAAAGUGCUGACAGUGCAGAUA	UAUCUGCACUGUCAGCACUUUA
hsa-miR-107	AGCAGCAUUGUACAGGGCUAUC	GAUAGCCCUGUACAAUGCUGCU
hsa-miR-10b	UACCCUGUAGAACCGAAUUUGU	ACAAAUUCGGUUCUACAGGGUA
hsa-miR-128b	UCACAGUGAACCGGUCUCUUUC	GAAAGAGACCGGUUCACUGUGA
hsa-miR-130b	CAGUGCAAUGAUGAAAGGGCAU	AUGCCCUUUCAUCAUUGCACUG
hsa-miR-140-3p	UACCACAGGGUAGAACCACGGA	UCCGUGGUUCUACCCUGUGGUA
hsa-miR-142-5p	CCCAUAAAGUAGAAAGCACUAC	GUAGUGCUUUCUACUUUAUGGG
hsa-miR-151-5p	UCGAGGAGCUCACAGUCUAGUA	UACUAGACUGUGAGCUCCUCGA
hsa-miR-155	UUAAUGCUAAUCGUGAUAGGGG	CCCCUAUCACGAUUAGCAUUAA
hsa-miR-181a	AACAUUCAACGCUGUCGGUGAG	CUCACCGACAGCGUUGAAUGUU
hsa-miR-181b	AACAUUCAUUGCUGUCGGUGGG	CCCACCGACAGCAAUGAAUGUU
hsa-miR-181c	AACAUUCAACCUGUCGGUGAGU	ACUCACCGACAGGUUGAAUGUU
hsa-miR-182	UUUGGCAAUGGUAGAACUCACA	UGUGAGUUCUACCAUUGCCAAA
hsa-miR-183	UAUGGCACUGGUAGAAUUCACU	AGUGAAUUCUACCAGUGCCAUA
hsa-miR-184	UGGACGGAGAACUGAUAAGGGU	ACCCUUAUCAGUUCUCCGUCCA
hsa-miR-185	UGGAGAGAAAGGCAGUUCCUGA	UCAGGAACUGCCUUUCUCUCCA
hsa-miR-186	CAAAGAAUUCUCCUUUUGGGCU	AGCCCAAAAGGAGAAUUCUUUG
hsa-miR-187	UCGUGUCUUGUGUUGCAGCCGG	CCGGCUGCAACACAAGACACGA
hsa-miR-188-3p	CUCCCACAUGCAGGGUUUGCAG	CUGCAAACCCUGCAUGUGGGAG
hsa-miR-188-5p	CAUCCCUUGCAUGGUGGAGGGU	ACCCUCCACCAUGCAAGGGAUG
hsa-miR-189	GUGCCUACUGAGCUGAUAUCAG	CUGAUAUCAGCUCAGUAGGCAC
hsa-miR-190	UGAUAUGUUUGAUAUAUUAGGU	ACCUAAUAUAUCAAACAUAUCA
hsa-miR-191	CAACGGAAUCCCAAAAGCAGCU	AGCUGCUUUUGGGAUUCCGUUG
hsa-miR-192	CUGACCUAUGAAUUGACAGCCA	UGGCUGUCAAUUCAUAGGUCAG
hsa-miR-193-3p	AACUGGCCUACAAAGUCCCAGU	ACUGGGACUUUGUAGGCCAGUU
hsa-miR-193-5p	UGGGUCUUUGCGGGCAAGAUGA	UCAUCUUGCCCGCAAAGACCCA
hsa-miR-194	UGUAACAGCAACUCCAUGUGGA	UCCACAUGGAGUUGCUGUUACA
hsa-miR-195	UAGCAGCACAGAAAUAUUGGCA	UGCCAAUAUUUCUGUGCUGCUA
hsa-miR-196	UAGGUAGUUUCAUGUUGUUGGG	CCCAACAACAUGAAACUACCUA
hsa-miR-197	UUCACCACCUUCUCCACCCAGC	GCUGGGUGGAGAAGGUGGUGAA
hsa-miR-198	GGUCCAGAGGGGAGAUAGGUUC	GAACCUAUCUCCCCUCUGGACC
hsa-miR-199a-3p	ACAGUAGUCUGCACAUUGGUUA	UAACCAAUGUGCAGACUACUGU
hsa-miR-199a-5p	CCCAGUGUUCAGACUACCUGUU	AACAGGUAGUCUGAACACUGGG

microRNA name	microRNA sequence (5' to 3')	Anti-microRNA molecule sequence (5' to 3')
hsa-miR-199b	CCCAGUGUUUAGACUAUCUGUU	AACAGAUAGUCUAAACACUGGG
hsa-miR-200a	UAACACUGUCUGGUAACGAUGU	ACAUCGUUACCAGACAGUGUUA
hsa-miR-200b	CUCUAAUACUGCCUGGUAAUGA	UCAUUACCAGGCAGUAUUAGAG
hsa-miR-200c	AAUACUGCCGGGUAAUGAUGGA	UCCAUCAUUACCCGGCAGUAUU
hsa-miR-203	GUGAAAUGUUUAGGACCACUAG	CUAGUGGUCCUAAACAUUUCAC
hsa-miR-204	UUCCCUUUGUCAUCCUAUGCCU	AGGCAUAGGAUGACAAAGGGAA
hsa-miR-205	UCCUUCAUUCCACCGGAGUCUG	CAGACUCCGGUGGAAUGAAGGA
hsa-miR-206	UGGAAUGUAAGGAAGUGUGUGG	CCACACACUUCCUUACAUUCCA
hsa-miR-208	AUAAGACGAGCAAAAAGCUUGU	ACAAGCUUUUUGCUCGUCUUAU
hsa-miR-210	CUGUGCGUGUGACAGCGGCUGA	UCAGCCGCUGUCACACGCACAG
hsa-miR-211	UUCCCUUUGUCAUCCUUCGCCU	AGGCGAAGGAUGACAAAGGGAA
hsa-miR-212	UAACAGUCUCCAGUCACGGCCA	UGGCCGUGACUGGAGACUGUUA
hsa-miR-213	ACCAUCGACCGUUGAUUGUACC	GGUACAAUCAACGGUCGAUGGU
hsa-miR-214	ACAGCAGGCACAGACAGGCAGU	ACUGCCUGUCUGUGCCUGCUGU
hsa-miR-215	AUGACCUAUGAAUUGACAGACA	UGUCUGUCAAUUCAUAGGUCAU
hsa-miR-216	UAAUCUCAGCUGGCAACUGUGA	UCACAGUUGCCAGCUGAGAUUA
hsa-miR-217	UACUGCAUCAGGAACUGAUUGG	CCAAUCAGUUCCUGAUGCAGUA
hsa-miR-218	UUGUGCUUGAUCUAACCAUGUG	CACAUGGUUAGAUCAAGCACAA
hsa-miR-219	UGAUUGUCCAAACGCAAUUCUU	AAGAAUUGCGUUUGGACAAUCA
hsa-miR-220	CCACACCGUAUCUGACACUUUG	CAAAGUGUCAGAUACGGUGUGG
hsa-miR-221	AGCUACAUUGUCUGCUGGGUUU	AAACCCAGCAGACAAUGUAGCU
hsa-miR-222	AGCUACAUCUGGCUACUGGGUC	GACCCAGUAGCCAGAUGUAGCU
hsa-miR-223	UGUCAGUUUGUCAAAUACCCCA	UGGGGUAUUUGACAAACUGACA
hsa-miR-224	CAAGUCACUAGUGGUUCCGUUU	AAACGGAACCACUAGUGACUUG
hsa-miR-28-5p	AAGGAGCUCACAGUCUAUUGAG	CUCAAUAGACUGUGAGCUCCUU
hsa-miR-290	CUCAAACUGUGGGGGCACUUUC	GAAAGUGCCCCCACAGUUUGAG
hsa-miR-296	AGGGCCCCCCCUCAAUCCUGUU	AACAGGAUUGAGGGGGGGCCCU
hsa-miR-299	UGGUUUACCGUCCCACAUACAU	AUGUAUGUGGGACGGUAAACCA
hsa-miR-301	CAGUGCAAUAGUAUUGUCAAAG	CUUUGACAAUACUAUUGCACUG
hsa-miR-302	UAAGUGCUUCCAUGUUUUGGUG	CACCAAAACAUGGAAGCACUUA
hsa-miR-30e	UGUAAACAUCCUUGACUGGAAG	CUUCCAGUCAAGGAUGUUUACA
hsa-miR-320	AAAAGCUGGGUUGAGAGGGCGA	UCGCCCUCUCAACCCAGCUUUU
hsa-miR-321	UAAGCCAGGGAUUGUGGGUUCG	CGAACCCACAAUCCCUGGCUUA
hsa-miR-322	AAACAUGAAUUGCUGCUGUAUC	GAUACAGCAGCAAUUCAUGUUU
hsa-miR-323	GCACAUUACACGGUCGACCUCU	AGAGGUCGACCGUGUAAUGUGC
hsa-miR-324-3p	CCACUGCCCCAGGUGCUGCUGG	CCAGCAGCACCUGGGGCAGUGG
hsa-miR-324-5p	CGCAUCCCCUAGGGCAUUGGUG	CACCAAUGCCCUAGGGGAUGCG
hsa-miR-326	CCUCUGGGCCCUUCCUCCAGCC	GGCUGGAGGAAGGGCCCAGAGG
hsa-miR-328	CUGGCCCUCUCUGCCCUUCCGU	ACGGAAGGGCAGAGAGGGCCAG
hsa-miR-329	AACACACCCAGCUAACCUUUUU	AAAAAGGUUAGCUGGGUGUGUU
hsa-miR-34a	UGGCAGUGUCUUAGCUGGUUGU	ACAACCAGCUAAGACACUGCCA
hsa-miR-34b	AGGCAGUGUCAUUAGCUGAUUG	CAAUCAGCUAAUGACACUGCCU
hsa-miR-34c	AGGCAGUGUAGUUAGCUGAUUG	CAAUCAGCUAACUACACUGCCU
hsa-miR-92	UAUUGCACUUGUCCCGGCCUGU	ACAGGCCGGGACAAGUGCAAUA
hsa-miR-93	AAAGUGCUGUUCGUGCAGGUAG	CUACCUGCACGAACAGCACUUU
hsa-miR-95	UUCAACGGGUAUUUAUUGAGCA	UGCUCAAUAAAUACCCGUUGAA
hsa-miR-96	UUUGGCACUAGCACAUUUUUGC	GCAAAAAUGUGCUAGUGCCAAA
hsa-miR-98	UGAGGUAGUAAGUUGUAUUGUU	AACAAUACAACUUACUACCUCA
mmu-miR-106a	CAAAGUGCUAACAGUGCAGGUA	UACCUGCACUGUUAGCACUUUG
mmu-miR-10b	CCCUGUAGAACCGAAUUUGUGU	ACACAAAUUCGGUUCUACAGGG
mmu-miR-135b	UAUGGCUUUUCAUUCCUAUGUG	CACAUAGGAAUGAAAAGCCAUA

microRNA name	microRNA sequence (5' to 3')	Anti-microRNA molecule sequence (5' to 3')
mmu-miR-148b	UCAGUGCAUCACAGAACUUUGU	ACAAAGUUCUGUGAUGCACUGA
mmu-miR-151-3p	CUAGACUGAGGCUCCUUGAGGA	UCCUCAAGGAGCCUCAGUCUAG
mmu-miR-155	UUAAUGCUAAUUGUGAUAGGGG	CCCCUAUCACAAUUAGCAUUAA
mmu-miR-199b	CCCAGUGUUUAGACUACCUGUU	AACAGGUAGUCUAAACACUGGG
mmu-miR-200b	UAAUACUGCCUGGUAAUGAUGA	UCAUCAUUACCAGGCAGUAUUA
mmu-miR-203	UGAAAUGUUUAGGACCACUAGA	UCUAGUGGUCCUAAACAUUUCA
mmu-miR-211	UUCCCUUUGUCAUCCUUUGCCU	AGGCAAAGGAUGACAAAGGGAA
mmu-miR-217	UACUGCAUCAGGAACUGACUGG	CCAGUCAGUUCCUGAUGCAGUA
mmu-miR-224	UAAGUCACUAGUGGUUCCGUUU	AAACGGAACCACUAGUGACUUA
mmu-miR-28-3p	CACUAGAUUGUGAGCUGCUGGA	UCCAGCAGCUCACAAUCUAGUG
mmu-miR-290	CUCAAACUAUGGGGGCACUUUU	AAAAGUGCCCCCAUAGUUUGAG
mmu-miR-291-3p	AAAGUGCUUCCACUUUGUGUGC	GCACACAAAGUGGAAGCACUUU
mmu-miR-291-5p	CAUCAAAGUGGAGGCCCUCUCU	AGAGAGGGCCUCCACUUUGAUG
mmu-miR-292-3p	AAGUGCCGCCAGGUUUUGAGUG	CACUCAAAACCUGGCGGCACUU
mmu-miR-292-5p	ACUCAAACUGGGGGCUCUUUUG	CAAAAGAGCCCCCAGUUUGAGU
mmu-miR-293	AGUGCCGCAGAGUUUGUAGUGU	ACACUACAAACUCUGCGGCACU
mmu-miR-294	AAAGUGCUUCCCUUUUGUGUGU	ACACACAAAAGGGAAGCACUUU
mmu-miR-295	AAAGUGCUACUACUUUUGAGUC	GACUCAAAAGUAGUAGCACUUU
mmu-miR-297	AUGUAUGUGUGCAUGUGCAUGU	ACAUGCACAUGCACACAUACAU
mmu-miR-298	GGCAGAGGAGGGCUGUUCUUCC	GGAAGAACAGCCCUCCUCUGCC
mmu-miR-300	UAUGCAAGGGCAAGCUCUCUUC	GAAGAGAGCUUGCCCUUGCAUA
mmu-miR-31	AGGCAAGAUGCUGGCAUAGCUG	CAGCUAUGCCAGCAUCUUGCCU
mmu-miR-322	AAACAUGAAGCGCUGCAACACC	GGUGUUGCAGCGCUUCAUGUUU
mmu-miR-325	CCUAGUAGGUGCUCAGUAAGUG	CACUUACUGAGCACCUACUAGG
mmu-miR-326	CCUCUGGGCCCUUCCUCCAGUC	GACUGGAGGAAGGGCCCAGAGG
mmu-miR-330	GCAAAGCACAGGGCCUGCAGAG	CUCUGCAGGCCCUGUGCUUUGC
mmu-miR-331	GCCCCUGGGCCUAUCCUAGAAC	GUUCUAGGAUAGGCCCAGGGGC
mmu-miR-337	UUCAGCUCCUAUAUGAUGCCUU	AAGGCAUCAUAUAGGAGCUGAA
mmu-miR-338	UCCAGCAUCAGUGAUUUUGUUG	CAACAAAAUCACUGAUGCUGGA
mmu-miR-339	UCCCUGUCCUCCAGGAGCUCAC	GUGAGCUCCUGGAGGACAGGGA
mmu-miR-340	UCCGUCUCAGUUACUUUAUAGC	GCUAUAAAGUAACUGAGACGGA
mmu-miR-341	UCGAUCGGUCGGUCGGUCAGUC	GACUGACCGACCGACCGAUCGA
mmu-miR-342	UCUCACACAGAAAUCGCACCCG	CGGGUGCGAUUUCUGUGUGAGA
mmu-miR-344	UGAUCUAGCCAAAGCCUGACUG	CAGUCAGGCUUUGGCUAGAUCA
mmu-miR-345	UGCUGACCCCUAGUCCAGUGCU	AGCACUGGACUAGGGGUCAGCA
mmu-miR-346	UGUCUGCCCGAGUGCCUGCCUC	GAGGCAGGCACUCGGGCAGACA
mmu-miR-34b	UAGGCAGUGUAAUUAGCUGAUU	AAUCAGCUAAUUACACUGCCUA
mmu-miR-350	UUCACAAAGCCCAUACACUUUC	GAAAGUGUAUGGGCUUUGUGAA
mmu-miR-351	UCCCUGAGGAGCCCUUUGAGCC	GGCUCAAAGGGCUCCUCAGGGA
mmu-miR-7b	UGGAAGACUUGUGAUUUUGUUG	CAACAAAAUCACAAGUCUUCCA
mmu-miR-92	UAUUGCACUUGUCCCGGCCUGA	UCAGGCCGGGACAAGUGCAAUA
mmu-miR-93	CAAAGUGCUGUUCGUGCAGGUA	UACCUGCACGAACAGCACUUUG
rno-miR-327	CCUUGAGGGGCAUGAGGGUAGU	ACUACCCUCAUGCCCCUCAAGG
rno-miR-333	GUGGUGUGCUAGUUACUUUUGG	CCAAAAGUAACUAGCACACCAC
rno-miR-335	UCAAGAGCAAUAACGAAAAAUG	CAUUUUUCGUUAUUGCUCUUGA
rno-miR-336	UCACCCUUCCAUAUCUAGUCUC	GAGACUAGAUAUGGAAGGGUGA
rno-miR-343	UCUCCCUCCGUGUGCCCAGUAU	AUACUGGGCACACGGAGGGAGA
rno-miR-347	UGUCCCUCUGGGUCGCCCAGCU	AGCUGGGCGACCCAGAGGGACA
rno-miR-349	CAGCCCUGCUGUCUUAACCUCU	AGAGGUUAAGACAGCAGGGCUG
rno-miR-352	AGAGUAGUAGGUUGCAUAGUAC	GUACUAUGCAACCUACUACUCU

Table 2: Novel Human microRNA and anti-microRNA sequences.

microRNA name	microRNA sequence (5' to 3')	Anti-microRNA molecule sequence (5' to 3')
hsa-miR-361	UUAUCAGAAUCUCCAGGGGUAC	GUACCCCUGGAGAUUCUGAUAA
hsa-miR-362	AAUCCUUGGAACCUAGGUGUGA	UCACACCUAGGUUCCAAGGAUU
hsa-miR-363	AUUGCACGGUAUCCAUCUGUAA	UUACAGAUGGAUACCGUGCAAU
hsa-miR-364	CGGCGGGGACGGCGAUUGGUCC	GGACCAAUCGCCGUCCCCGCCG
hsa-miR-365	UAAUGCCCCUAAAAAUCCUUAU	AUAAGGAUUUUUAGGGGCAUUA
hsa-miR-366	UAACUGGUUGAACAACUGAACC	GGUUCAGUUGUUCAACCAGUUA

Table 3: C. elegans microRNA and anti-microRNA sequences.

microRNA name	microRNA sequence (5' to 3')	Anti-microRNA molecule sequence (5' to 3')
Cel-let-7	UGAGGUAGUAGGUUGUAUAGUU	AACUAUACAACCUACUACCUCA
Cel-lin-4	UCCCUGAGACCUCAAGUGUGAG	CUCACACUUGAGGUCUCAGGGA
Cel-miR-1	UGGAAUGUAAAGAAGUAUGUAG	CUACAUACUUCUUUACAUUCCA
Cel-miR-2	UAUCACAGCCAGCUUUGAUGUG	CACAUCAAAGCUGGCUGUGAUA
Cel-miR-34	AGGCAGUGUGGUUAGCUGGUUG	CAACCAGCUAACCACACUGCCU
Cel-miR-35	UCACCGGGUGGAAACUAGCAGU	ACUGCUAGUUUCCACCCGGUGA
Cel-miR-36	UCACCGGGUGAAAAUUCGCAUG	CAUGCGAAUUUUCACCCGGUGA
Cel-miR-37	UCACCGGGUGAACACUUGCAGU	ACUGCAAGUGUUCACCCGGUGA
Cel-miR-38	UCACCGGGAGAAAAACUGGAGU	ACUCCAGUUUUUCUCCCGGUGA
Cel-miR-39	UCACCGGGUGUAAAUCAGCUUG	CAAGCUGAUUUACACCCGGUGA
Cel-miR-40	UCACCGGGUGUACAUCAGCUAA	UUAGCUGAUGUACACCCGGUGA
Cel-miR-41	UCACCGGGUGAAAAAUCACCUA	UAGGUGAUUUUUCACCCGGUGA
Cel-miR-42	CACCGGGUUAACAUCUACAGAG	CUCUGUAGAUGUUAACCCGGUG
Cel-miR-43	UAUCACAGUUUACUUGCUGUCG	CGACAGCAAGUAAACUGUGAUA
Cel-miR-44	UGACUAGAGACACAUUCAGCUU	AAGCUGAAUGUGUCUCUAGUCA
Cel-miR-45	UGACUAGAGACACAUUCAGCUU	AAGCUGAAUGUGUCUCUAGUCA
Cel-miR-46	UGUCAUGGAGUCGCUCUCUUCA	UGAAGAGAGCGACUCCAUGACA
Cel-miR-47	UGUCAUGGAGGCGCUCUCUUCA	UGAAGAGAGCGCCUCCAUGACA
Cel-miR-48	UGAGGUAGGCUCAGUAGAUGCG	CGCAUCUACUGAGCCUACCUCA
Cel-miR-49	AAGCACCACGAGAAGCUGCAGA	UCUGCAGCUUCUCGUGGUGCUU
Cel-miR-50	UGAUAUGUCUGGUAUUCUUGGG	CCCAAGAAUACCAGACAUAUCA
Cel-miR-51	UACCCGUAGCUCCUAUCCAUGU	ACAUGGAUAGGAGCUACGGGUA
Cel-miR-52	CACCCGUACAUAUGUUUCCGUG	CACGGAAACAUAUGUACGGGUG
Cel-miR-53	CACCCGUACAUUUGUUUCCGUG	CACGGAAACAAAUGUACGGGUG
Cel-miR-54	UACCCGUAAUCUUCAUAAUCCG	CGGAUUAUGAAGAUUACGGGUA
Cel-miR-55	UACCCGUAUAAGUUUCUGCUGA	UCAGCAGAAACUUAUACGGGUA
Cel-miR-56	UACCCGUAAUGUUUCCGCUGAG	CUCAGCGGAAACAUUACGGGUA
Cel-miR-57	UACCCUGUAGAUCGAGCUGUGU	ACACAGCUCGAUCUACAGGGUA
Cel-miR-58	UGAGAUCGUUCAGUACGGCAAU	AUUGCCGUACUGAACGAUCUCA
Cel-miR-59	UCGAAUCGUUUAUCAGGAUGAU	AUCAUCCUGAUAAACGAUUCGA
Cel-miR-60	UAUUAUGCACAUUUUCUAGUUC	GAACUAGAAAAUGUGCAUAAUA
Cel-miR-61	UGACUAGAACCGUUACUCAUCU	AGAUGAGUAACGGUUCUAGUCA
Cel-miR-62	UGAUAUGUAAUCUAGCUUACAG	CUGUAAGCUAGAUUACAUAUCA
Cel-miR-63	AUGACACUGAAGCGAGUUGGAA	UUCCAACUCGCUUCAGUGUCAU

microRNA name	microRNA sequence (5' to 3')	Anti-microRNA molecule sequence (5' to 3')
Cel-miR-64	UAUGACACUGAAGCGUUACCGA	UCGGUAACGCUUCAGUGUCAUA
Cel-miR-65	UAUGACACUGAAGCGUAACCGA	UCGGUUACGCUUCAGUGUCAUA
Cel-miR-66	CAUGACACUGAUUAGGGAUGUG	CACAUCCCUAAUCAGUGUCAUG
Cel-miR-67	UCACAACCUCCUAGAAAGAGUA	UACUCUUUCUAGGAGGUUGUGA
Cel-miR-68	UCGAAGACUCAAAAGUGUAGAC	GUCUACACUUUUGAGUCUUCGA
Cel-miR-69	UCGAAAAUUAAAAAGUGUAGAA	UUCUACACUUUUUAAUUUUCGA
Cel-miR-70	UAAUACGUCGUUGGUGUUUCCA	UGGAAACACCAACGACGUAUUA
Cel-miR-71	UGAAAGACAUGGGUAGUGAACG	CGUUCACUACCCAUGUCUUUCA
Cel-miR-72	AGGCAAGAUGUUGGCAUAGCUG	CAGCUAUGCCAACAUCUUGCCU
Cel-miR-73	UGGCAAGAUGUAGGCAGUUCAG	CUGAACUGCCUACAUCUUGCCA
Cel-miR-74	UGGCAAGAAAUGGCAGUCUACA	UGUAGACUGCCAUUUCUUGCCA
Cel-miR-75	UUAAAGCUACCAACCGGCUUCA	UGAAGCCGGUUGGUAGCUUUAA
Cel-miR-76	UUCGUUGUUGAUGAAGCCUUGA	UCAAGGCUUCAUCAACAACGAA
Cel-miR-77	UUCAUCAGGCCAUAGCUGUCCA	UGGACAGCUAUGGCCUGAUGAA
Cel-miR-78	UGGAGGCCUGGUUGUUUGUGCU	AGCACAAACAACCAGGCCUCCA
Cel-miR-79	AUAAAGCUAGGUUACCAAAGCU	AGCUUUGGUAACCUAGCUUUAU
Cel-miR-227	AGCUUUCGACAUGAUUCUGAAC	GUUCAGAAUCAUGUCGAAAGCU
Cel-miR-80	UGAGAUCAUUAGUUGAAAGCCG	CGGCUUUCAACUAAUGAUCUCA
Cel-miR-81	UGAGAUCAUCGUGAAAGCUAGU	ACUAGCUUUCACGAUGAUCUCA
Cel-miR-82	UGAGAUCAUCGUGAAAGCCAGU	ACUGGCUUUCACGAUGAUCUCA
Cel-miR-83	UAGCACCAUAUAAAUUCAGUAA	UUACUGAAUUUAUAUGGUGCUA
Cel-miR-84	UGAGGUAGUAUGUAAUAUUGUA	UACAAUAUUACAUACUACCUCA
Cel-miR-85	UACAAAGUAUUUGAAAAGUCGU	ACGACUUUUCAAAUACUUUGUA
Cel-miR-86	UAAGUGAAUGCUUUGCCACAGU	ACUGUGGCAAAGCAUUCACUUA
Cel-miR-87	GUGAGCAAAGUUUCAGGUGUGC	GCACACCUGAAACUUUGCUCAC
Cel-miR-90	UGAUAUGUUGUUUGAAUGCCCC	GGGGCAUUCAAACAACAUAUCA
Cel-miR-124	UAAGGCACGCGGUGAAUGCCAC	GUGGCAUUCACCGCGUGCCUUA
Cel-miR-228	AAUGGCACUGCAUGAAUUCACG	CGUGAAUUCAUGCAGUGCCAUU
Cel-miR-229	AAUGACACUGGUUAUCUUUUCC	GGAAAAGAUAACCAGUGUCAUU
Cel-miR-230	GUAUUAGUUGUGCGACCAGGAG	CUCCUGGUCGCACAACUAAUAC
Cel-miR-231	UAAGCUCGUGAUCAACAGGCAG	CUGCCUGUUGAUCACGAGCUUA
Cel-miR-232	UAAAUGCAUCUUAACUGCGGUG	CACCGCAGUUAAGAUGCAUUUA
Cel-miR-233	UUGAGCAAUGCGCAUGUGCGGG	CCCGCACAUGCGCAUUGCUCAA
Cel-miR-234	UUAUUGCUCGAGAAUACCCUUU	AAAGGGUAUUCUCGAGCAAUAA
Cel-miR-235	UAUUGCACUCUCCCCGGCCUGA	UCAGGCCGGGGAGAGUGCAAUA
Cel-miR-236	UAAUACUGUCAGGUAAUGACGC	GCGUCAUUACCUGACAGUAUUA
Cel-miR-237	UCCCUGAGAAUUCUCGAACAGC	GCUGUUCGAGAAUUCUCAGGGA
Cel-miR-238	UUUGUACUCCGAUGCCAUUCAG	CUGAAUGGCAUCGGAGUACAAA
Cel-miR-239a	UUUGUACUACACAUAGGUACUG	CAGUACCUAUGUGUAGUACAAA
Cel-miR-239b	UUUGUACUACACAAAAGUACUG	CAGUACUUUUGUGUAGUACAAA
Cel-miR-240	UACUGGCCCCCAAAUCUUCGCU	AGCGAAGAUUUGGGGGCCAGUA
Cel-miR-241	UGAGGUAGGUGCGAGAAAUGAC	GUCAUUUCUCGCACCUACCUCA
Cel-miR-242	UUGCGUAGGCCUUUGCUUCGAG	CUCGAAGCAAAGGCCUACGCAA
Cel-miR-243	CGGUACGAUCGCGGCGGGAUAU	AUAUCCCGCCGCGAUCGUACCG
Cel-miR-244	UCUUUGGUUGUACAAAGUGGUA	UACCACUUUGUACAACCAAAGA
Cel-miR-245	AUUGGUCCCCUCCAAGUAGCUC	GAGCUACUUGGAGGGGACCAAU
Cel-miR-246	UUACAUGUUUCGGGUAGGAGCU	AGCUCCUACCCGAAACAUGUAA
Cel-miR-247	UGACUAGAGCCUAUUCUCUUCU	AGAAGAGAAUAGGCUCUAGUCA
Cel-miR-248	UACACGUGCACGGAUAACGCUC	GAGCGUUAUCCGUGCACGUGUA
Cel-miR-249	UCACAGGACUUUUGAGCGUUGC	GCAACGCUCAAAAGUCCUGUGA
Cel-miR-250	UCACAGUCAACUGUUGGCAUGG	CCAUGCCAACAGUUGACUGUGA

microRNA name	microRNA sequence (5' to 3')	Anti-microRNA molecule sequence (5' to 3')
Cel-miR-251	UUAAGUAGUGGUGCCGCUCUUA	UAAGAGCGGCACCACUACUUAA
Cel-miR-252	UAAGUAGUAGUGCCGCAGGUAA	UUACCUGCGGCACUACUACUUA
Cel-miR-253	CACACCUCACUAACACUGACCA	UGGUCAGUGUUAGUGAGGUGUG
Cel-miR-254	UGCAAAUCUUUCGCGACUGUAG	CUACAGUCGCGAAAGAUUUGCA
Cel-miR-256	UGGAAUGCAUAGAAGACUGUAC	GUACAGUCUUCUAUGCAUUCCA
Cel-miR-257	GAGUAUCAGGAGUACCCAGUGA	UCACUGGGUACUCCUGAUACUC
Cel-miR-258	GGUUUUGAGAGGAAUCCUUUUA	UAAAAGGAUUCCUCUCAAAACC
Cel-miR-259	AGUAAAUCUCAUCCUAAUCUGG	CCAGAUUAGGAUGAGAUUUACU
Cel-miR-260	GUGAUGUCGAACUCUUGUAGGA	UCCUACAAGAGUUCGACAUCAC
Cel-miR-261	UAGCUUUUUAGUUUUCACGGUG	CACCGUGAAAACUAAAAAGCUA
Cel-miR-262	GUUUCUCGAUGUUUUCUGAUAC	GUAUCAGAAAACAUCGAGAAAC
Cel-miR-264	GGCGGGUGGUUGUUGUUAUGGG	CCCAUAACAACAACCACCCGCC
Cel-miR-265	UGAGGGAGGAAGGGUGGUAUUU	AAAUACCACCCUUCCUCCCUCA
Cel-miR-266	AGGCAAGACUUUGGCAAAGCUU	AAGCUUUGCCAAAGUCUUGCCU
Cel-miR-267	CCCGUGAAGUGUCUGCUGCAAU	AUUGCAGCAGACACUUCACGGG
Cel-miR-268	GGCAAGAAUUAGAAGCAGUUUG	CAAACUGCUUCUAAUUCUUGCC
Cel-miR-269	GGCAAGACUCUGGCAAAACUUG	CAAGUUUUGCCAGAGUCUUGCC
Cel-miR-270	GGCAUGAUGUAGCAGUGGAGAU	AUCUCCACUGCUACAUCAUGCC
Cel-miR-271	UCGCCGGGUGGGAAAGCAUUCG	CGAAUGCUUUCCCACCCGGCGA
Cel-miR-272	UGUAGGCAUGGGUGUUUGGAAG	CUUCCAAACACCCAUGCCUACA
Cel-miR-273	UGCCCGUACUGUGUCGGCUGCU	AGCAGCCGACACAGUACGGGCA

Table 4: Drosophila microRNA and anti-microRNA sequences.

microRNA name	microRNA sequence (5' to 3')	Anti-microRNA molecule sequence (5' to 3')
Dme-miR-263a	GUUAAUGGCACUGGAAGAAUUC	GAAUUCUUCCAGUGCCAUUAAC
Dme-miR-184	UGGACGGAGAACUGAUAAGGGC	GCCCUUAUCAGUUCUCCGUCCA
Dme-miR-274	UUUUGUGACCGACACUAACGGG	CCCGUUAGUGUCGGUCACAAAA
Dme-miR-275	UCAGGUACCUGAAGUAGCGCGC	GCGCGCUACUUCAGGUACCUGA
Dme-miR-92a	CAUUGCACUUGUCCCGGCCUAU	AUAGGCCGGGACAAGUGCAAUG
Dme-miR-219	UGAUUGUCCAAACGCAAUUCUU	AAGAAUUGCGUUUGGACAAUCA
Dme-miR-276a	UAGGAACUUCAUACCGUGCUCU	AGAGCACGGUAUGAAGUUCCUA
Dme-miR-277	UAAAUGCACUAUCUGGUACGAC	GUCGUACCAGAUAGUGCAUUUA
Dme-miR-278	UCGGUGGGACUUUCGUCCGUUU	AAACGGACGAAAGUCCCACCGA
Dme-miR-133	UUGGUCCCCUUCAACCAGCUGU	ACAGCUGGUUGAAGGGGACCAA
Dme-miR-279	UGACUAGAUCCACACUCAUUAA	UUAAUGAGUGUGGAUCUAGUCA
Dme-miR-33	AGGUGCAUUGUAGUCGCAUUGU	ACAAUGCGACUACAAUGCACCU
Dme-miR-280	UGUAUUUACGUUGCAUAUGAAA	UUUCAUAUGCAACGUAAAUACA
Dme-miR-281	UGUCAUGGAAUUGCUCUCUUUG	CAAAGAGAGCAAUUCCAUGACA
Dme-miR-282	AAUCUAGCCUCUACUAGGCUUU	AAAGCCUAGUAGAGGCUAGAUU
Dme-miR-283	UAAAUAUCAGCUGGUAAUUCUG	CAGAAUUACCAGCUGAUAUUUA
Dme-miR-284	UGAAGUCAGCAACUUGAUUCCA	UGGAAUCAAGUUGCUGACUUCA
Dme-miR-34	UGGCAGUGUGGUUAGCUGGUUG	CAACCAGCUAACCACACUGCCA
Dme-miR-124	UAAGGCACGCGGUGAAUGCCAA	UUGGCAUUCACCGCGUGCCUUA
Dme-miR-79	UAAAGCUAGAUUACCAAAGCAU	AUGCUUUGGUAAUCUAGCUUUA
Dme-miR-276b	UAGGAACUUAAUACCGUGCUCU	AGAGCACGGUAUUAAGUUCCUA
Dme-miR-210	UUGUGCGUGUGACAGCGGCUAU	AUAGCCGCUGUCACACGCACAA
Dme-miR-285	UAGCACCAUUCGAAAUCAGUGC	GCACUGAUUUCGAAUGGUGCUA
Dme-miR-100	AACCCGUAAAUCCGAACUUGUG	CACAAGUUCGGAUUUACGGGUU
Dme-miR-92b	AAUUGCACUAGUCCCGGCCUGC	GCAGGCCGGGACUAGUGCAAUU
Dme-miR-286	UGACUAGACCGAACACUCGUGC	GCACGAGUGUUCGGUCUAGUCA
Dme-miR-287	UGUGUUGAAAAUCGUUUGCACG	CGUGCAAACGAUUUUCAACACA
Dme-miR-87	UUGAGCAAAAUUUCAGGUGUGU	ACACACCUGAAAUUUUGCUCAA
Dme-miR-263b	CUUGGCACUGGGAGAAUUCACA	UGUGAAUUCUCCCAGUGCCAAG
Dme-miR-288	UUUCAUGUCGAUUUCAUUUCAU	AUGAAAUGAAAUCGACAUGAAA
Dme-miR-289	UAAAUAUUUAAGUGGAGCCUGC	GCAGGCUCCACUUAAAUAUUUA
Dme-bantam	UGAGAUCAUUUUGAAAGCUGAU	AUCAGCUUUCAAAAUGAUCUCA
Dme-miR-303	UUUAGGUUUCACAGGAAACUGG	CCAGUUUCCUGUGAAACCUAAA
Dme-miR-31b	UGGCAAGAUGUCGGAAUAGCUG	CAGCUAUUCCGACAUCUUGCCA
Dme-miR-304	UAAUCUCAAUUUGUAAAUGUGA	UCACAUUUACAAAUUGAGAUUA
Dme-miR-305	AUUGUACUUCAUCAGGUGCUCU	AGAGCACCUGAUGAAGUACAAU
Dme-miR-9c	UCUUUGGUAUUCUAGCUGUAGA	UCUACAGCUAGAAUACCAAAGA
Dme-miR-306	UCAGGUACUUAGUGACUCUCAA	UUGAGAGUCACUAAGUACCUGA
Dme-miR-9b	UCUUUGGUGAUUUUAGCUGUAU	AUACAGCUAAAAUCACCAAAGA
Dme-miR-125	UCCCUGAGACCCUAACUUGUGA	UCACAAGUUAGGGUCUCAGGGA
Dme-miR-307	UCACAACCUCCUUGAGUGAGCG	CGCUCACUCAAGGAGGUUGUGA
Dme-miR-308	AAUCACAGGAUUAUACUGUGAG	CUCACAGUAUAAUCCUGUGAUU
dme-miR-31a	UGGCAAGAUGUCGGCAUAGCUG	CAGCUAUGCCGACAUCUUGCCA
dme-miR-309	GCACUGGGUAAAGUUUGUCCUA	UAGGACAAACUUUACCCAGUGC
dme-miR-310	UAUUGCACACUUCCCGGCCUUU	AAAGGCCGGGAAGUGUGCAAUA
dme-miR-311	UAUUGCACAUUCACCGGCCUGA	UCAGGCCGGUGAAUGUGCAAUA
dme-miR-312	UAUUGCACUUGAGACGGCCUGA	UCAGGCCGUCUCAAGUGCAAUA
dme-miR-313	UAUUGCACUUUUCACAGCCCGA	UCGGGCUGUGAAAAGUGCAAUA
dme-miR-314	UAUUCGAGCCAAUAAGUUCGG	CCGAACUUAUUGGCUCGAAUA

microRNA name	microRNA sequence (5' to 3')	Anti-microRNA molecule sequence (5' to 3')
dme-miR-315	UUUUGAUUGUUGCUCAGAAAGC	GCUUUCUGAGCAACAAUCAAAA
dme-miR-316	UGUCUUUUUCCGCUUACUGGCG	CGCCAGUAAGCGGAAAAAGACA
dme-miR-317	UGAACACAGCUGGUGGUAUCCA	UGGAUACCACCAGCUGUGUUCA
dme-miR-318	UCACUGGGCUUUGUUUAUCUCA	UGAGAUAAACAAAGCCCAGUGA
dme-miR-2c	UAUCACAGCCAGCUUUGAUGGG	CCCAUCAAAGCUGGCUGUGAUA
Dme-miR-iab45p	ACGUAUACUGAAUGUAUCCUGA	UCAGGAUACAUUCAGUAUACGU
Dme-miR-iab43p	CGGUAUACCUUCAGUAUACGUA	UACGUAUACUGAAGGUAUACCG

EXAMPLES

Example 1: Materials and Methods

Oligonucleotide synthesis

MiR-21 were synthesized using 5'-silyl, 2'-ACE phosphoramidites (Dharmacon, Lafayette, CO, USA) on 0.2 μmol synthesis columns using a modified ABI 394 synthesizer (Foster City, CA, USA) (Scaringe, Methods Enzymol. *317*, 3-18 (2001) and Scaringe, Methods *23*, 206-217 (2001)). The phosphate methyl group was removed by flushing the column with 2 ml of 0.2 M 2-carbamoyl-2-cyanoethylene-1,1-dithiolate trihydrate in DMF/water (98:2 v/v) for 30 min at room temperature. The reagent was removed and the column rinsed with 10 ml water followed by 10 ml acetonitrile. The oligonucleotide was cleaved and eluted from the solid support by flushing with 1.6 ml of 40% aqueous methylamine over 2 min, collected in a screwcap vial and incubated for 10 min at 55 °C. Subsequently, the base-treated oligonucleotide was dried down in an Eppendorf concentrator to remove methylamine and water. The residue was dissolved in sterile 2'-deprotection buffer (400 μl of 100 mM acetate-TEMED, pH 3.8, for a 0.2 μmol scale synthesis) and incubated for 30 minutes at 60 °C to remove the 2' ACE group. The oligoribonucleotide was precipitated from the acetate-TEMED solution by adding 24 μl 5 M NaCl and 1.2 ml of absolute ethanol.

2'-O-Methyl oligoribonucleotides were synthesized using 5'-DMT, 2'-O-methyl phosphoramidites (Proligo, Hamburg, Germany) on 1 μmol synthesis columns loaded with 3'-aminomodifier (TFA) C7 Icaa control pore glass support (Chemgenes, MA, USA). The aminolinker was added in order to also use the oligonucleotides for conjugation to amino group

reactive reagents, such as biotin succinimidyl esters. The synthesis products were deprotected for 16 h at 55 °C in 30% aqueous ammonia and then precipitated by the addition of 12 ml absolute 1-butanol. The full-length product was then gel-purified using a denaturing 20% polyacrylamide gel. 2'-Deoxyoligonucleotides were prepared using 0.2 μmol scale synthesis and standard DNA synthesis reagents (Proligo, Hamburg, Germany).

The sequences of the 2'-O-methyl oligoribonucleotides were 5'-GUCAACAUCAGUCUGAUAAGCUAL (L, 3' aminolinker) for 2'-OMe miR-21, and 5'-AAGGCAAGCUGACCCUGAAGUL for EGFP 2'-OMe antisense, 5'-UGAAGUCCCAGUCGAACGGAAL for EGFP 2'-OMe reverse; the sequence of chimeric 2'-OMe/DNA oligonucleotides was 5'-GTCAACATCAGTCTGATAAGCTA<u>GCG</u>L for 2'-deoxy miR-21 (underlined, 2'-OMe residues), and 5'-AAGGCAAGCTGACCCTGAAGT<u>GCG</u>L for EGFP 2'-deoxy antisense.

The miR-21 cleavage substrate was prepared by PCR-based extension of the partially complementary synthetic DNA oligonucleotides 5'-GAACAATTGCTTTTACAGATGCACATATCGAGGTGAACATCACGTACGTCAACATCAGTCTGATAAGCTATCGGTTGGCAGAAGCTAT and 5'-GGCATAAAGAATTGAAGAGAGTTTTCACTGCATACGACGATTCTGTGATTTGTATTCAGCCCATATCGTTTCATAGCTTCTGCCAACCGA. The extended dsDNA was then used as template for a new PCR with primers 5'-TAATACGACTCACTATAGAACAATTGCTTTTACAG and 5'-ATTTAGGTGACACTATAGGCATAAAGAATTGAAGA to introduce the T7 and SP6 promoter sequences for in vitro transcription. The PCR product was ligated into pCR2.1-TOPO (Invitrogen). Plasmids isolated from sequence-verified clones were used as templates for PCR to produce sufficient template for run-off in vitro transcription reactions using phage RNA polymerases (Elbashir et al., EMBO *20,* 6877-6888 (2001)). ^{32}P-Cap-labelling was performed as reported (Martinez et al., Cell *110,* 563-574 (2002)).

Plasmids

Plasmids pEGFP-S-21 and pEGFP-A-21 were generated by T4 DNA ligation of preannealed oligodeoxynucleotides 5'-GGCCTCAACATCAGTCTGATAAGCTAGGTACCT

and 5'-GGCCAGGTACCTAGCTTATCAGACTGATGTTGA into NotI digested pEGFP-N-1 (Clontech). The plasmid pHcRed-C1 was from Clontech.

HeLa extracts and miR-21 quantification

HeLa cell extracts were prepared as described (Dignam et al., Nucleic Acid Res. *11* 1475-1489 (1983)). $5x10^9$ cells from HeLa suspension cultures were collected by centrifugation and washed with PBS (pH7.4). The cell pellet (approx. 15 ml) was re-suspended in two times of its volume with 10mM KCl/1.5 mM $MgCl_2$/0.5 mM dithiothreitol/10mM HEPES-KOH (pH 7.9) and homogenized by douncing. The nuclei were then removed by centrifugation of the cell lysate at 1000 g for 10 min. The supernatant was spun in an ultracentrifuge for 1 h at 10,5000 g to obtain the cytoplasmic S100 extract. The concentration of KCl of the S100 extract was subsequently raised to 100 mM by the addition of 1 M KCl. The extract was then supplemented with 10% glycerol and frozen in liquid nitrogen.

280 μg of total RNA was isolated from 1 ml of S100 extract using the acidic guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski et al., Anal. Biochem. *162,* 156-159 (1987)). A calibration curve for miR-21 Northern signals was produced by loading increasing amounts (10 to 30000 pg) of synthetically made miR-21 (Lim et al. et al., Genes & Devel. *17,* 991-1008 (2003)). Northern blot analysis was performed as described using 30 μg of total RNA per well (Lagos-Quintana et al., Science *294,* 853-858 (2001)).

In vitro miRNA cleavage and inhibition assay

2'-O-Methyl oligoribonucleotides or 2'-deoxyoligonucleotides were pre-incubated with HeLa S100 at 30 °C for 20 min prior to the addition of the cap-labeled miR-21 target RNA. The concentration of the reaction components were 5 nM target RNA, 1 mM ATP, 0.2 mM GTP, 10 U/ml RNasin (Promega) and 50% HeLa S100 extract in a final reaction volume of 25 μl. The reaction time was 1.5 h at 30 °C. The reaction was stopped by addition of 200 μl of 300 mM NaCl/25 mM EDTA/20% w/v SDS/200 mM Tris HCl (pH7.5). Subsequently, proteinase K was added to a final concentration of 0.6 mg/ml and the sample was incubated for 15 min at 65 °C. After phenol/chloroform extraction, the RNA was ethanol-precipitated and separated on a 6% denaturing polyacrylamide gel. Radioactivity was detected by phosphorimaging.

Cell culture and transfection

HeLa S3 and HeLa S3/GFP were grown in 5% CO2 at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 unit/ml penicillin, and 100 µg/ml streptomycin. One day before transfection, 105 cells were plated in 500 µl DMEM containing 10% FBS per well of a 24-well plate. Plasmid and plasmid/oligonucleotide transfection was carried out with Lipofectamine2000 (Invitrogen). 0.2 µg pEGFP or its derivatives were cotransfected with 0.3 µg pHcRed with or without 10 pmol of 2'-O-methyl oligoribonucleotide or 10 pmol of 2'-deoxyoligonucleotide per well. Fluorescent cell images were recorded on a Zeiss Axiovert 200 inverted fluorescence microscope (Plan-Apochromat 10x/0.45) equipped with Chroma Technology Corp. filter sets 41001 (EGFP) and 41002c (HcRed) and AxioVision 3.1 software.

Example 2: MicroRNA-21 Cleavage of Target RNA

In order to assess the ability of modified oligonucleotides to specifically interfere with miRNA function, we used our previously described mammalian biochemical system developed for assaying RISC activity (Martinez et al., Cell *100,* 563-574 (2002)). Zamore and colleagues (Hutvágner et al., Science *297,* 2056-2050 (2002)) showed that crude cytoplasmic cell lysates and eIF2C2 immunoprecipitates prepared from these lysates contain let-7 RNPs that specifically cleave let-7-complementary target RNAs. We previously reported that in HeLa cells, numerous miRNAs are expressed including several let-7 miRNA variants (Lagos-Quintana et al., Science *294,* 853-858 (2001)).

To assess if other HeLa cell miRNAs are also engaged in RISC like miRNPs we examined the cleavage of a 32P-cap-labelled substrate RNA with a complementary site to the highly expressed miR-21 (Lagos-Quintana et al., Science *294,* 853-858 (2001); Mourelatos et al., Genes & Dev. *16,* 720-728 (2002)). Sequence-specific target RNA degradation was readily observed and appeared to be approximately 2- to 5-fold more effective than cleavage of a similar let-7 target RNA (Figure 2A, lane 1, and data not shown). We therefore decided to interfere with miR-21 guided target RNA cleavage.

Example 3: Anti MicroRNA-21 2'-O-methyl Oligoribonucleotide Inhibited MicroRNA-21-Induced Cleavage of Target RNA

A 24-nucleotide 2'-O-methyl oligoribonucleotide that contained a 3' C7 aminolinker and was complementary to the longest form of the miR-21 was synthesized. The aminolinker was introduced in order to enable post-synthetic conjugation of non-nucleotidic residues such as biotin.

Increasing concentrations of anti miR-21 2'-O-methyl oligoribonucleotide and a control 2'-O-methyl oligoribonucleotide cognate to an EGFP sequence were added to the S100 extract 20 min prior to the addition of 32P-cap-labelled substrate. We determined the concentration of miR-21 in the S100 extract by quantitative Northern blotting to be 50 pM (Lim et al., Genes & Devel. *17,* 991-1008 (2003)).

The control EGFP oligonucleotide did not interfere with miR-21 cleavage even at the highest applied concentration (Figure 2A, lanes 2-3). In contrast, the activity of miR-21 was completely blocked at a concentration of only 3 nM (Figure 2A, lane 5), and a concentration of 0.3 nM showed a substantial 60%-70% reduction of cleavage activity (Figure 2, lane 6). At a concentration of 0.03 nM, the cleavage activity of miR-21 was not affected when compared to the lysate alone (Figure 2, lane 1, 7).

Antisense 2'-deoxyoligonucleotides (approximately 90% DNA molecules) at concentrations identical to those of 2'-O-methyl oligoribonucleotides, we could not detect blockage of miR-21 induced cleavage (Figure 2A, lanes 8-10). The 2'-deoxynucleotides used in this study were protected against 3'-exonucleases by the addition of three 2'-O-methyl ribonucleotide residues.

Example 4: Anti MicroRNA-21 2'-O-methyl Oligoribonucleotide Inhibited MicroRNA-21-Induced Cleavage of Target RNA *In Vitro*

In order to monitor the activity of miR-21 in HeLa cells, we constructed reporter plasmids that express EGFP mRNA that contains in its 3' UTR a 22-nt sequence complementary to miR-21 (pEGFP-S-21) or in sense orientation to miR-21 (p-EGFP-A-21). Endogenous miRNAs have previously been shown to act like siRNAs by cleaving reporter mRNAs carrying

sequences perfectly complementary to miRNA. To monitor transfection efficiency and specific interference with the EGFP indicator plasmids, the far-red fluorescent protein encoding plasmid pHcRed-C1 was cotransfected.

Expression of EGFP was observed in HeLa cells transfected with pEGFP and pEGFP-A-21 (Figure 3, rows 1 and 2), but not from those transfected with pEGFP-S-21 (Figure 3, row 3). However, expression of EGFP from pEGFP-S-21 was restored upon cotransfection with anti miR-21 2'-O-methyl oligoribonucleotide (Figure 3, row 4). Consistent with our above observation, the 2'-deoxy anti miR-21 oligonucleotide showed no effect (Figure 3, row 5). Similarly, cotransfection of the EGFP 2'-O-methyl oligoribonucleotide in sense orientation with respect to the EGFP mRNA (or antisense to EGFP guide siRNA) had no effect (Figure 3,row 6).

We have demonstrated that miRNP complexes can be effectively and sequence-specifically inhibited with 2'-O-methyl oligoribonucleotides antisense to the guide strand positioned in the RNA silencing complex.